



# Genome-wide localization of Rrm3 and Pif1 DNA helicases at stalled active and inactive DNA replication forks of *Saccharomyces cerevisiae*



Silvia Emma Rossi<sup>a,b</sup>, Walter Carotenuto<sup>a</sup>, Michele Giannattasio<sup>a,b,\*</sup>

<sup>a</sup> IFOM (Fondazione Istituto FIRC di Oncologia Molecolare) via Adamello 16, 20139 Milan, Italy

<sup>b</sup> Università degli Studi di Milano, Dipartimento di Bioscienze, Via Celoria 26, 20133 Milan, Italy

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## ABSTRACT

The genome of the budding yeast *Saccharomyces cerevisiae* is sequenced and the location and dynamic of activation of DNA replication origins are known. G1 synchronized yeast cells can be released into S-phase in the presence of hydroxyurea (HU) (1), which slows down DNA replication and retains replication forks in proximity of DNA replication origins. In this condition, the Chromatin Immuno-Precipitation on chip (ChIP on chip) (2–4) of replisome components allows the precise localization of all active DNA replication forks. This analysis can be coupled with the ssDNA-BromodeoxyUridine (ssDNA-BrdU) Immuno-Precipitation on chip (ssDNA-BrdU IP on chip) technique (5–7), which detects the location of newly synthesized DNA. Comparison of binding and BrdU incorporation profiles allows to locate a factor of interest at DNA replication forks genome wide. We present datasets deposited in the gene expression omnibus (GEO) database under accession number GSE68214, which show how the DNA helicases Rrm3 and Pif1 (8) associate to active and inactive DNA replication forks.

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Specifications	
Organism/cell line/tissue	<i>S. cerevisiae</i>
Connected publication	Rossi et al. Cell Reports 2015 Oct 6;13(1):80–92
Sequencer or array type	[Sc03b_MR] Affymetrix GeneChip <i>S. cerevisiae</i> Tiling 1.0R Array
Data format	CEL, bar
Experimental factors	DNA polymerase $\alpha$ , Rrm3 and Pif1.BrdU Incorporation.
Experimental features	ChIP on chip and ssDNA-BrdU IP on chip experiments.
Consent	Reuse and publication of datasets under GSE68214 accession number is under authorization of FIRC institute of molecular Oncology foundation (IFOM), Milano, Italy.
Sample source location	FIRC institute of molecular Oncology foundation (IFOM), Milano, Italy.

## 1. Direct link to deposited data

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE68214>.

## 2. Experimental design, materials and methods

Indicated yeast strains (see GSE68214 and [9]), were synchronized in G1 with 4  $\mu$ g/ml of  $\alpha$ -factor at 28 °C for 2 h in YP + 2% glucose and

released in S-phase in the presence of HU (150 mM [1]) and, when required for the analysis of newly synthesized DNA, BrdU (200–500  $\mu$ g/ml) [5–7]. ChIP on chip experiments in the GSE68214 series were conducted as described [2–4] with the following modifications: zirconium beads were used for cell breakage and amplified DNA with the whole genome amplification kit (WGA-SIGMA) has been purified using the Qiagen QIAquick PCR purification kit instead of the YM130 cartridges [3]. The amplification steps in the ChIP on chip protocol were conducted in non-saturating conditions and the amount of DNA used to hybridize the affymetrix chips was normalized to 4  $\mu$ g within the different samples to preserve quantitative ratios [3]. Briefly, CEL files obtained by scanning of the hybridized affymetrix chips were analyzed using a modified version of the Tiling Array Suite software (TAS) from affymetrix. The software does a linear scale normalization of input CEL files (IP and Sup) intensity so that the median value is equal to a selected target intensity of 500. Signals and the p-value changes obtained from TAS per each probe position were subsequently used by the software to detect clusters of enriched signals as ranges within the chromosomes. Conditions for clusters detection in whole range (at least 600 bps), except for segments within the range shorter than 600 bps, were: log2 signal (IP/SUP binding ratio) positive and change in p-value (evaluated using Wilcoxon signed rank test) <0.2 [2]. Specifically engineered yeast strains capable of incorporating BrdU in to the DNA [10,11] have been used to conduct ssDNA-BrdU IP on chip experiments [5–7]. BrdU incorporation profiles have been generated as described for protein binding

\* Corresponding author.

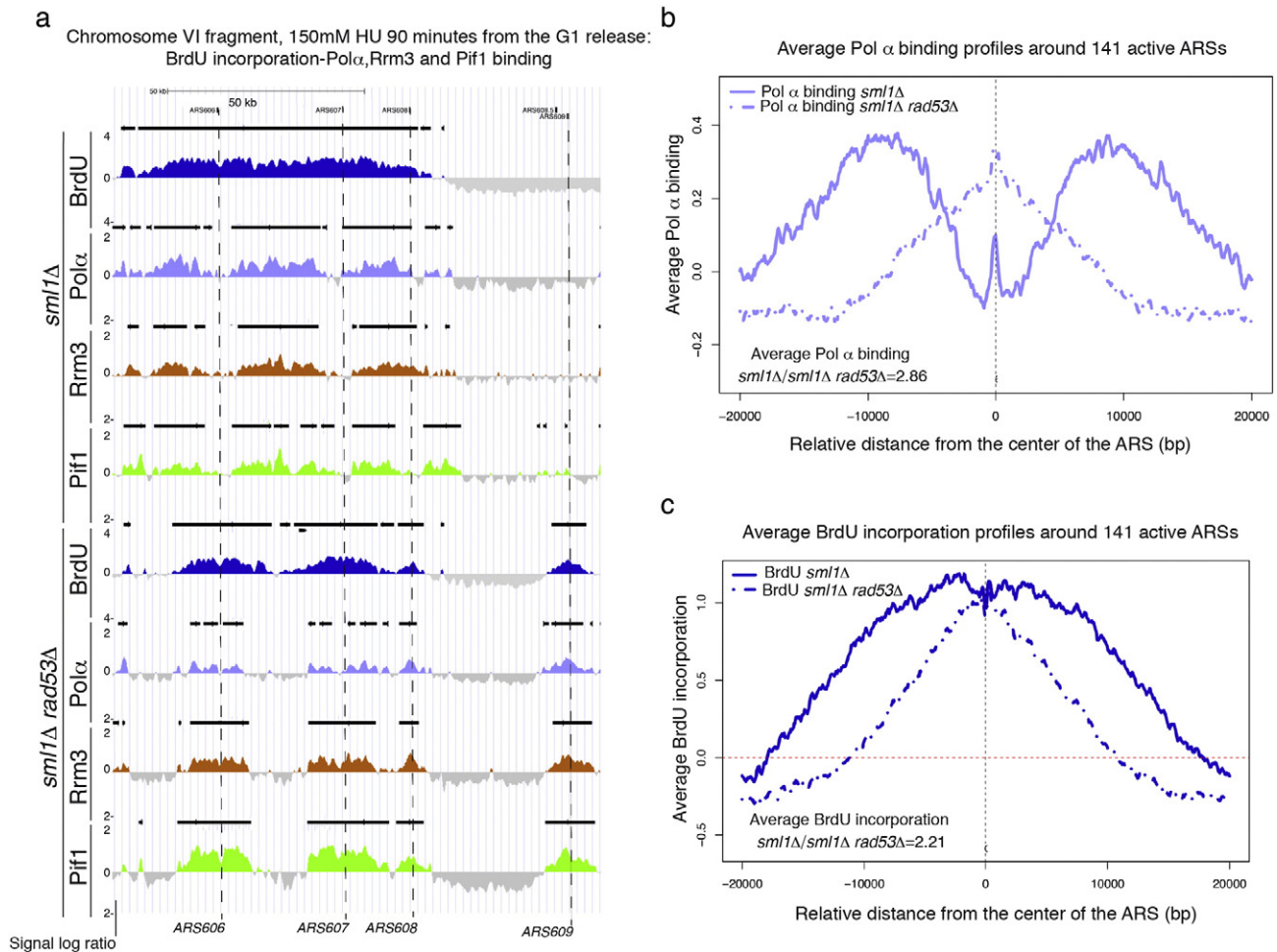
E-mail address: [michele.giannattasio@ifom.eu](mailto:michele.giannattasio@ifom.eu) (M. Giannattasio).

profiles. Genome wide binding and BrdU incorporation profiles can be superimposed and the statistical significance of binding and BrdU cluster overlappings can be calculated using a confrontation against a null hypothesis model generated with a Montecarlo-like simulation [2]. Average profiling of DNA binding and BrdU incorporation signals within specific genomic *loci* can be obtained using sitepro script of CEAS package (Cis-Regulatory Element Annotation System) [12]. Briefly, log<sub>2</sub> signal (IP/SUP binding ratio) bed files obtained from protein binding and BrdU incorporation analysis were wig converted and used to draw average signals around 141 active DNA replication origins (Autonomously Replicating Sequences, ARSs), setting 50 bps as the profiling resolution and 20 kbps as the size of flanking regions from the center of each ARS. For the calculation of average binding or BrdU incorporation signals, negative values were set to zero. Total average binding or BrdU incorporation signals around 141 ARSs have been derived as average of the average of signals from the 50 bp bins created by the sitepro CEAS script where negative values were set to zero.

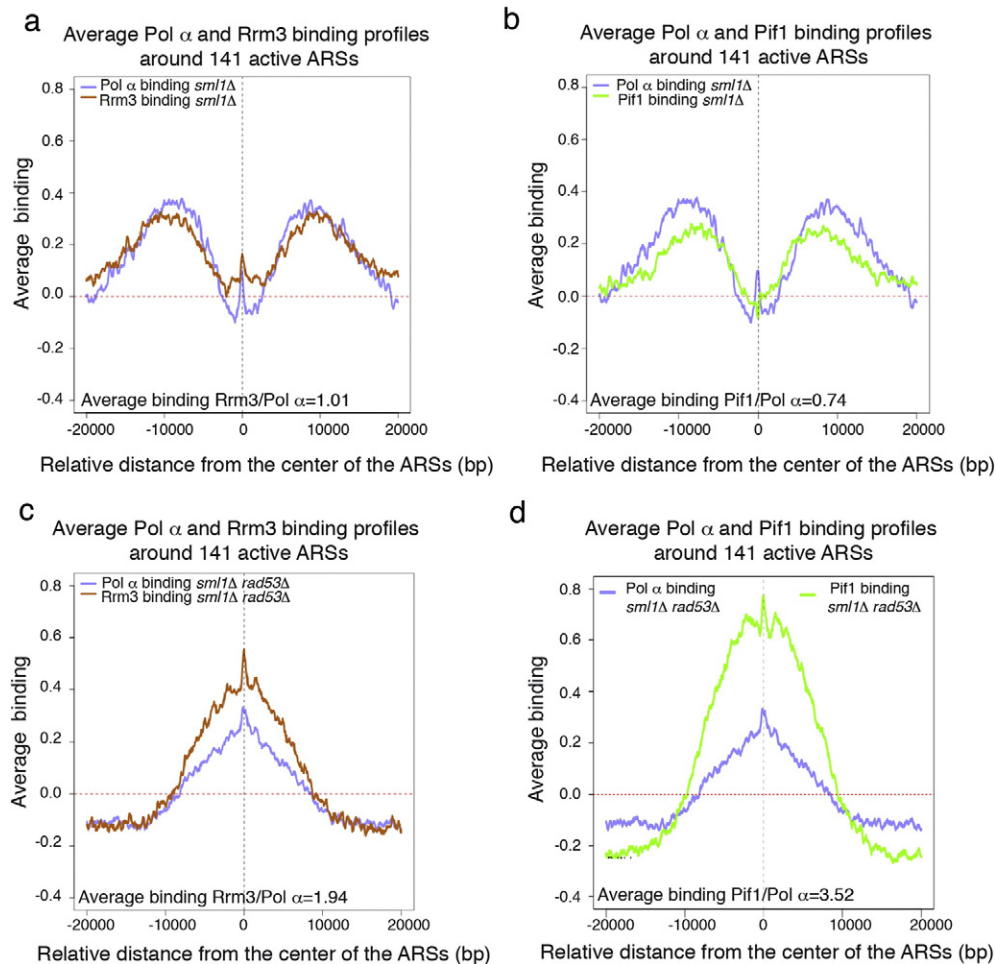
### 3. Results

In Fig. 1a, DNA polymerase  $\alpha$  (Pol  $\alpha$ ), Rrm3, Pif1 [8] binding and BrdU incorporation clusters were determined in *sml1* $\Delta$  (control strain)

and *sml1* $\Delta$  *rad53* $\Delta$  cells released from G1 into S-phase in the presence of 150 mM of HU for 90 min. Pol  $\alpha$  binding clusters overlapped with Rrm3 and Pif1 clusters in the two strains in a statistically significant way, suggesting either that Rrm3 and Pif1 are replisome-replication fork components in this experimental condition or that the absence of RAD53 does not influence the distribution of their binding sites to the forks (Fig. 1a). Interestingly, binding clusters of Pol  $\alpha$ , Rrm3 and Pif1 localized to the borders of the BrdU clusters surrounding active DNA replication origins in *sml1* $\Delta$  cells as expected for proteins, which move with the DNA replication forks (Fig. 1a–c and 2a, b). *rad53* $\Delta$  cells fire late and dormant DNA replication origins in HU [13,14]. According to their localization at replication forks, Pif1 and Rrm3 binding clusters co-localized with Pol  $\alpha$  and BrdU clusters at the dormant origin ARS609, which is specifically fired in the absence of RAD53 in HU (Fig. 1a). RAD53 deletion inactivates DNA replication forks in the presence of HU leading either to dissociation of DNA polymerase  $\alpha$  from the DNA template or to the accumulation of aberrant cruciform DNA structures at fork branching points [9,15–17]. These fork-associated transitions strongly impair DNA replication fork progression in *rad53* cells treated with HU. Consistently, BrdU, Pol  $\alpha$ , Rrm3 and Pif1 clusters did not extend from the DNA replication origins in *rad53* cells treated with HU while they were more extended in the *sml1* $\Delta$  control cells (Fig. 1a–c and 2a–d). Accordingly,



**Fig. 1.** a) BrdU incorporation (dark blue) and Pol $\alpha$ -Flag (light blue), Rrm3-13Myc (brown) and Pif1-Flag (green) binding profiles were determined, respectively, by ssDNA-BrdU IP on chip and ChIP on chip in the strains CY12488, CY13284, CY12470, CY13074, CY12493, CY13282, CY12422 and CY13073 released from G1 into 150 mM of HU for 90 min [9]. The y-axis shows the enrichment signals expressed as ratio log<sub>2</sub> IP/SUP of *loci* significantly enriched in the IP fractions. The horizontal black bars above the picks indicate statistically significant BrdU or binding clusters. X-axis represents chromosomal coordinates. Early DNA replication origins (ARS606, ARS607) and the dormant origins (ARS608 and ARS609) are marked by dashed black lines. A black scale bar on the chromosome map indicates 50 kbps. b) Average binding profiles of DNA polymerase  $\alpha$  in *sml1* $\Delta$  and *sml1* $\Delta$  *rad53* $\Delta$  cells (from the experiment shown in panel a), in a window of 40 kbps centered on each of the 141 active ARSs are shown. The ratio of average Pol  $\alpha$  binding signals in *sml1* $\Delta$  versus *sml1* $\Delta$  *rad53* $\Delta$  cells is 2.86. c) Average BrdU incorporation profiles in *sml1* $\Delta$  and *sml1* $\Delta$  *rad53* $\Delta$  cells in a window of 40 kbps centered on 141 ARSs have been determined in the experiment shown in panel a. The ratio of BrdU incorporation signals in *sml1* $\Delta$  versus *sml1* $\Delta$  *rad53* $\Delta$  cells around 141 ARSs is 2.21.



**Fig. 2.** (a–d) Average binding profiles of the indicated proteins in *sml1Δ* (a, b) and *sml1Δ rad53Δ* cells (c, d) from the experiment shown in figure 1 panel a, in a window of 40 kbps centered on each of the 141 active ARSs are shown. Ratio of indicated total average binding signals is reported in each graph.

the average binding signal of DNA polymerase  $\alpha$  to a 40 kbps window centered on 141 active ARSs is 2.86 folds less in the absence of *RAD53* (Fig. 1b) and BrdU incorporation around the same ARSs is reduced of 2.21 folds without the checkpoint kinase (Fig. 1c). Moreover while Pol  $\alpha$ , Rrm3 and Pif1 binding clusters show overlapping bimodal distributions around 141 active ARSs in *sml1Δ* cells (Fig. 2a, b), consistent with forks moving away from the DNA replication origins, they remain close to the origin points in the absence of *RAD53* (Fig. 2c, d). These evidences strongly support the previously proposed idea that DNA replication forks do not proceed and progressively collapse in *rad53* cells treated with HU (Figs. 1a–c and 2a–d) [9,15–17]. Intriguingly, while Rrm3 and Pif1 bind with the same magnitude of Pol  $\alpha$  to 141 early ARSs in *sml1Δ* cells (Fig. 2a, b), the magnitude of their binding to the same ARSs is higher than Pol  $\alpha$  binding in *rad53* cells (Fig. 2c, d). These evidences suggest that additional substrates for Pif1 and Rrm3 may be created at collapsing forks of *rad53* cells treated with HU leading to an increased recruitment of Rrm3 and Pif1 at inactivated forks.

#### 4. Conclusions

DNA binding profiles of replisome–DNA replication fork components by ChIP on chip and BrdU incorporation profiles by ssDNA–BrdU IP on chip allow the precise localization of all active DNA replication forks in the genome of *Saccharomyces cerevisiae*. Superimposition analysis of binding and BrdU incorporation profiles (or profiles of other genome features) can be used to locate a factor of interest at active DNA replication forks or to study the relationships between DNA replication and other genome wide regulated processes. Calculation of average binding

or BrdU incorporation signals at specific genome *loci* in different genetic backgrounds allows uncovering roles in the regulation of specific chromosome processes. Ratio of total average binding signals provides a general indication of the relative strength of the binding of the considered factor to specific chromosome locations in different genetic backgrounds.

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